BIOTARGET

Clonogenic Survival Assay

Results

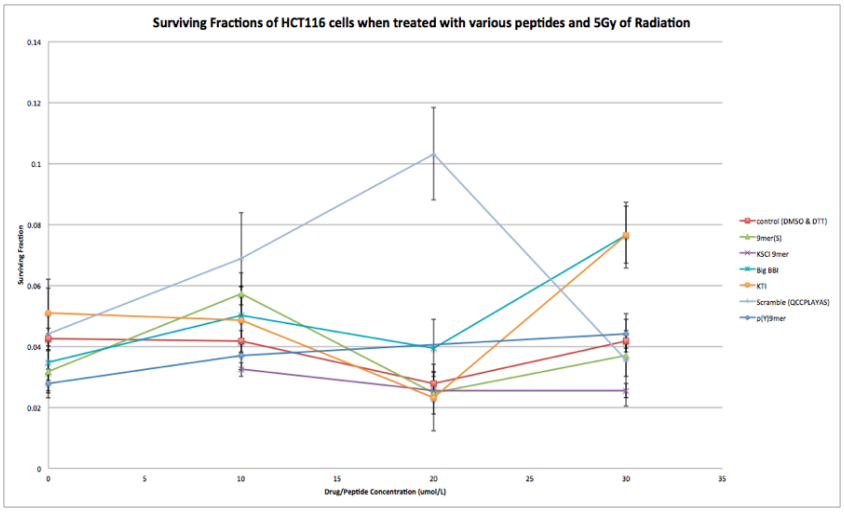

Figure 1. Surviving fraction of cells when irradiated at 5 Gy and treated with a peptide construct or a DMSO and DTT control

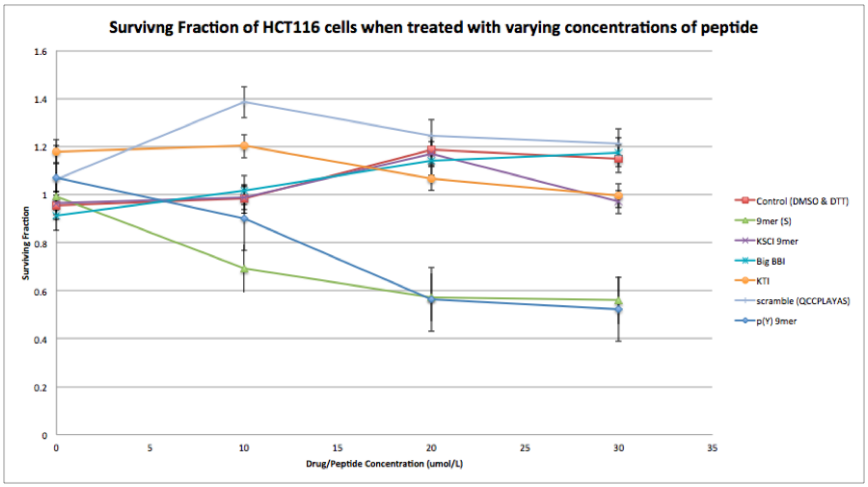

Figure 2. Surviving fraction of HCT116 cells when irradiated at 5 Gy and treated with ts respective peptide/control

Interpretation

The radio-protective effects of BBI on HCT 116 cells were examined using clonogenics assay. The viability of HCT116 cells was observed using this assay. Previous literature has shown that BBI exhibits radio-protective effects on cells that have a functional P53 gene, however our results do not support this assertion. The HCT116 cell line used in our study had a functional p53 gene, however no increase in survivability was observed. The study conducted had only 1 replicate however, so more replicates need

to be conducted to further verify this conclusion.

Project Achievements

Future Plans

More replicates need to be conducted in HCT116 in order to further examine the effects of BBI on cancer cell line. Another study needs to be conducted in different cell lines in order to examine the survivability effects of BBI, and whether the p53 gene is the only gene required for the radio-protective effect. A second clonogenics assay needs to be performed using primary fibroblast cells, in order to support the previous literature

Double Strand Break Assay using Immunofluorescence

Results

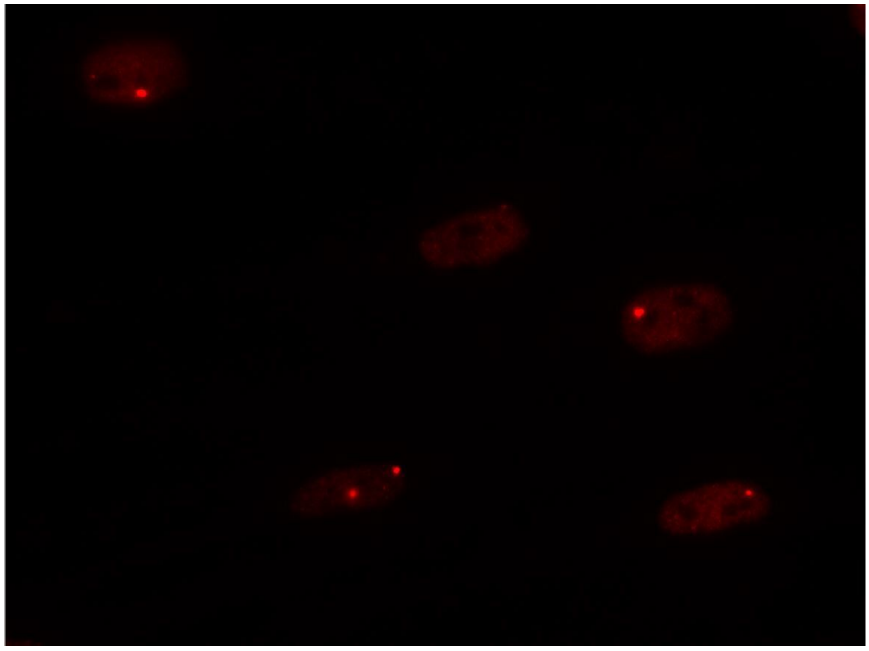

Figure 3. The Histology assay performed on primary fibroblast cells (1BR4) using primary mouse H2AX and rabbit 53BP1 antibody. The secondary antibody used was Alexa 488, Cy3 and DAPI. The cells were fixed at regular intervals. Only 1 replicate is shown.

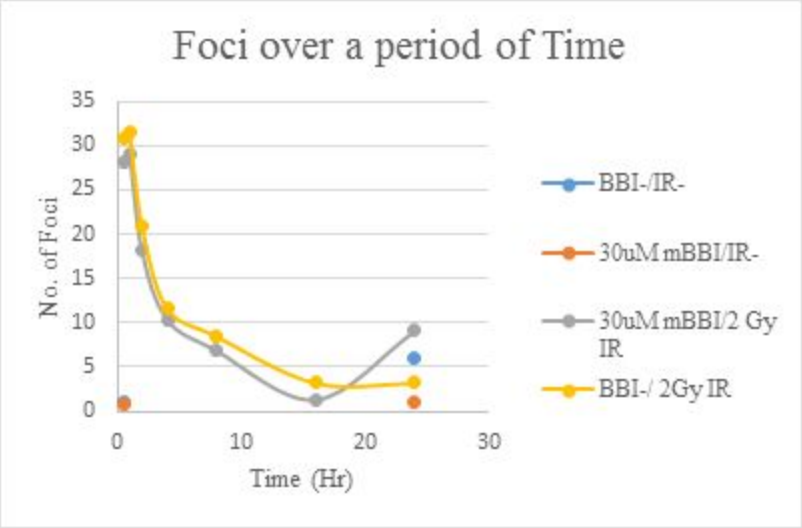

Fig 4. Line graph of foci counted during a y-H2AX assay run over 24 hours with 1BR3 human fibroblasts cells and radiation treatments labelled, incubation of BBI being the variable measurement in this assay.

Interpretation

The radio-protective effects of BBI were quantified using immunohistochemistry, where the number of DNA breaks were quantified after ionizing radiation dose and BBI administration. The radio-protective effects of oxidized or reduced peptide were examined and the graphs were plotted to analyze the differences. This experiment was conducted in order to elucidate whether the cysteine bridge present in BBI is necessary for its functioning, as a result only 2 time points (4hrs and 8hrs) were used since all the time points would have given us the same result. And it was found that there was no difference between the radio-protective effect of oxidized and reduced BBI. The number of double strand breaks repaired were fairly similar, at 4hrs and 8hrs time-point. (Note: The peptide was reduced with DDT). In order to elucidate the function and mechanism of BBI, a dosage study was also conducted with multiple IR dosage and multiple BBI concentration. The results were inconclusive however, since the inappropriate antibody was used. The staining was done using 53BP1, which is a protein that gets recruited in the later stages of the repair signal cascade, the 15-minute time point was not enough time for it to be tagged. The number of foci got capped at 25, since not all the protein was localized yet; as a result, we couldn't detect any effect of different doses of BBI. Since our dosage study was inconclusive, and we didn't have the resources or the time to run another assay, we used the previous literature to find the appropriate concentration. It has been shown in previous studies that 10-30uM of BBI is required for radioprotection, as a result we used 30uM for the histology assay. We found that BBI does have an effect; it reduces the number of foci

in nucleus, however, only 2 replicates are conducted yet.

Project Achievements

Future Plans

More studies need to be conducted in order to fully elucidate the mechanism and functioning of BBI using immunohistochemistry. The dosage study needs to be conducted with H2AX antibody instead of 53BP1, in order to eliminate the localization problem that occurred with 53BP1. H2AX is a phosphorylation marker, which happens quite quickly after DNA damage. The study conducted to differentiate between the two form of BBI (oxidized and reduced) can be repeated with more time points to ensure that the conclusion drawn is supported with more evidence.

Double Strand Break Assay using Immunofluorescence

Results

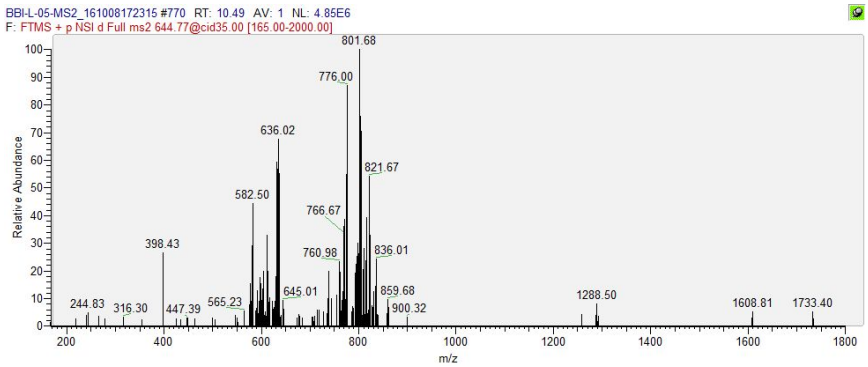

Figure 3. Mass Spectra of BBI in the +4 excited state

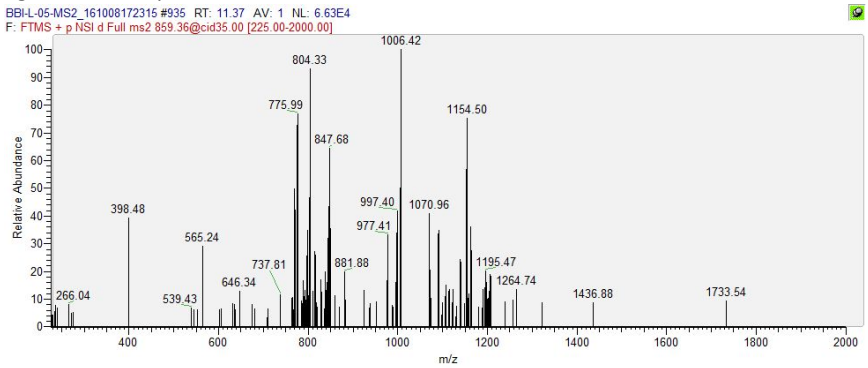

Figure 4. Mass Spectra of BBI in the +3 excited state

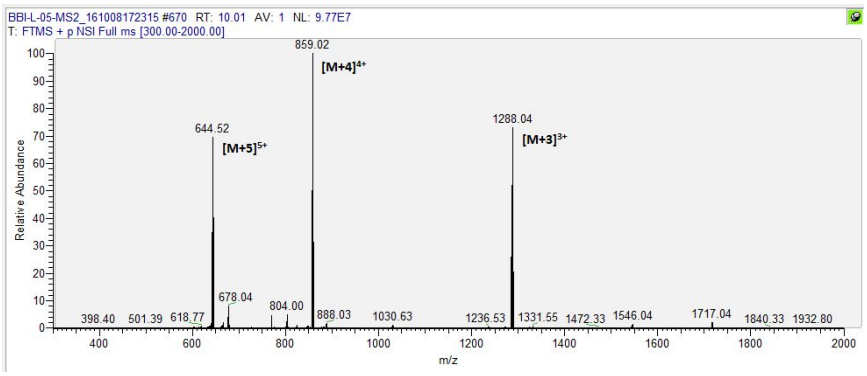

Figure 5. Standard mass spectra

Interpretation

During the mouse trials, blood was taken from mice at various time points to determine whether or not BBI with the TD-1 tag was able to migrate into the bloodstream. Unfortunately, when assayed with mass mass spectrometry there was no expected peptide construct found. Instead, we have chosen to publish the known peptide's spectra to aid future iGEM teams should they desire to work with TD-1/BBI

Project Achievements

BBI with the TD-1 tag was not found to be in any blood taken from the mice when assayed with mass mass spectrometry.

Future Plans

More studies need to be conducted in order to fully elucidate the mechanism and functioning of BBI with the TD-1 tag. It would be prudent to assay the TD-1 tag on its own, to observe whether it is the BBI attached to the tag that is disabling the transdermal function of the TD-1 tag.